Review Article

Clinical and laboratorial outcomes of xenogeneic biomaterials: literature review

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Background and Objective: Bone regeneration procedures are an important part of oral and maxillofacial surgery development, With the rising of dental implants research and techniques, bone augmentation became an essential also for oral implantology. The aim of this review was to , screen up the origins and the innovations through its laboratorial and clinical applications of xenografts.

Methods: A comprehensive search, without date limit was performed in MEDLINE, SCOPUS, Cochrane and Web of Science databases between November 2020 and March 2021, systematic reviews, human studies, clinical reports, *in vivo* and *in vitro* studies regarding history, application, outcomes or innovations in xenogeneic bone grafts usage were included, after initial search a manual cross-reference search was performed in the included papers.

Key Content and Findings: Xenogeneic grafts have been pointed as one of the most reliable alternatives to autogenous bone in augmentation techniques.

Conclusions: Xenogeneic grafts have been pointed as one of the most reliable alternatives to autogenous bone in augmentation techniques, but most of the applications were based on empirical expertise, and there is no consensus about its properties, fabrication methods and biological mechanisms which leads to clinical success. Clinical and pre-clinical research still necessary to comprehend the properties and gold standard applications of Xenografts.

Keywords: Xenogenous bone; xenografts, heterografts; bone substitutes; alveolar ridge augmentation

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Introduction

Tissue regeneration procedures are in the vanguard of medical science since the early 20th century, including bone regeneration procedures. The development of bone substitutes has a similar pathway with the development of bone surgery and osseointegrated implants. Regarding the precise indications, all grafting procedures present high rates of success and few complications (1-4).

Most of bone augmentation procedures use the autogenous bone as a source of biomaterial, and in the past years it has been considered a gold standard biomaterial for grafting procedures. The optimal bone graft substitute (gold

standard) material should have osteogenic, osteoinductive, osteoconductive and biocompatible properties, and the recipient must be provided with blood and nutrients. All these features have autogenous bone (5-7). Also, the ideal bone substitute must be biologically safe, demonstrating biocompatibility, reliable reproducibility and absence of toxicity (8).

Autogenous bone is generally the preferred graft material, it presents good results due to its osteoconductive, osteoinductive, and osteogenic properties and do not induce immunologic reactions (9-12). However, it demands technical ability, a second surgical site, and it is related to higher operative costs and donor-site complications, as the risk of donor's site deformation, risk of damage to anatomical structures, wound dehiscence and infection (11,13). To minimize the burden of a donor site creation, bone substitute materials have been extensively studied (2,14-16).

Bone substitute materials can be categorized in three groups: allogeneic grafts—from another individual within the same species; xenogeneic grafts—from another species; or alloplastic—synthetically produced grafts (17,18).

The xenogeneic bone graft has been indicated as an alternative to autologous bone, due its biocompatibility and osteoconductive properties (19-24). Xenogeneic grafts are defined as bone substitute materials derived from any non-human species.

Some of remarkable features of xenogeneic biomaterials are the osteoconductivity properties and the slow resorption rate that may contribute to maintaining tissue volume (25,26). The biocompatibility of xenogeneic materials of several species has been proved to be associated with the early encapsulation of bone substitute particles by a fibrous matrix, which will subsequently be used for bone conduction (26,27).

The aim of this review is to elucidate the technique and scientific development which lead to the actual state of art in xenogenous bone graft, and to stablish what are the new perspectives for its clinical use based on empirical and experimental data. We present the following article in accordance with the Narrative Review reporting checklist (available at https://fomm.amegroups.com/article/view/10.21037/fomm-21-43/rc).

Methods

This literature review was performed between November 2020 and March 2021, initial searches were performed in MEDLINE, SCOPUS, Cochrane and Web of Science databases, without date limit, in order to achieve the largest amount of studies. The words xenograft, xenogenous graft, heterograft, heterogenous graft, deproteinized bone, demineralized bone matrix, equine bone, bone substitutes, biomaterials and bone grafting, bone augmentation, augmentation, graft and bone healing were used alone and in combination. All studies, originally published in English or with available English version, and involving xenogeneic bone usage as clinical studies, *in vivo* and *in vitro* studies, systematic review, historical reviews and clinical reports were included. A manual cross-reference search was performed in included studies to identify suitable research not retrieved from initial search. Included papers were screened by abstract and full text by two independent reviewers, and disagreements were solved by an experienced reviewer.

Historic approach of xenogeneic biomaterials

Nowadays, one of the most used xenografts in dentistry is deproteinized bovine bone matrix (DBBM), but there are other natural sources of bone substitutes as the: caprine, equine and porcine bone matrix, also the exoskeleton of corals (6,28).

There is no precise starting point to the use of heterografts in the surgical practice. Some historical reports have identified Meek'en as the first surgeon to treat a bone defect in human with animal derived bone grafts, in the late XVII century, with the application of a dog bone graft to treat an injured Russian soldier (29), however the church at this time has condemned the practice and the graft must be removed. Two hundred years later Macewen (29,30) wrote its observations about the success of autografts and stimulated Senn to its experiments using oxen bone decalcified with chlorohydric acid (29,31). In the same time, Hamilton developed a concept that would lead the revolution in bone grafting, using sea sponges to treat open wounds he gave a huge contribution to the concept of a scaffold-based tissue growth (32).

In the early studies using xenogeneic bone transplants, it was expected that the mineral part could lead to antigenic reactions and that the organic part is able to carry the necessary stimuli to tissue growth (29,33,34). However, in 1937, Orell suggested that fat content carries the antigenic property in xenogeneic bone grafts and indicated the use of boiled animal bone as a useful material (35). Providing a material with porosity, controlled solubility, and mechanical resistance to compression (36). Two decades later, Hurley

et al. (1960) (37) resumed this hypothesis and stated the biologic failure of bone grafts is due the response evoked by organic constituents, which was previously demonstrated in the paper by Hurley and Losee (1957) (38).

At that point, it also known that the organic components, as collagen I and II and morphogenetic proteins are the key to osteoinduction in bone grafts, and mineralized grafts could only act in the osteoconductivity. However, the biomimetic and biomechanical properties of the deproteinized bone matrix have stimulated the development of new techniques of purification from organic components (39). Anderson *et al.* (39), in 1965, supported by favorable results in previous animal studies—which demonstrated that the treatment applied to the xenogeneic bone reduced the antigenicity—performed the first register of a clinical trial in humans using processed calf bone matrix, and proved that DBBM could be a reasonable alternative to graft procedures.

The use of DBBM in oral and maxillofacial surgery has far origins, but it was most highlighted after the increasing interest in dental implants science. In the early 80's the atrophic ridges were a challenge to dental implants placement, and bone augmentation procedures were highly demanded. At the same time, the annorganic bovine bone (ABB) has been used to other craniofacial applications, as the correction of calvaria defects. And further in the 90's the sinus lifting procedures increased the research about grafting materials, yet in 1996 Hürzeler *et al.* proved the heterogenous bone had enough osteoconductivity properties and was predictable and stable for maxillary augmentations (40).

However, at the same time the xenogeneic bone substitutes claim attention for its use, the synthetic hydroxyapatite has also emerged in the bone substitutes market. Some advantages of synthetic hydroxyapatite were the lower resorption rate and the high purity and homogeneity of the particles (41-43). Furthermore, some questions about the safety use of DBBM were raised, as the risk of Creutzfeldt-Jakob disease transmission through the presence of prion remnants in animal bone biomaterials (44,45). Bovine bone substitutes were claimed to have high risk of prion transmission, also goat and sheep (46). At this time equine bone has been introduced, with the possibility to supply in the same material a safe mineral and collagen matrix, which would not induce inflammatory encapsulation nor risk for disease transmission (47). However equine bone presented poor results in clinical trials, and new studies demonstrated the safety in the purification process of bovine bone matrix, considering minimal the risk of prion presence in physicochemical processed xenografts (48,49).

In the 90's and 2000 years, the research about oral and maxillofacial applications of xenografts had exponentially increased, most due to the achievements of bone augmentation procedures in the sinus lift techniques. Scheer and Boyne (50) in 1987 demonstrated the efficacy of ABB in extraction sockets, and in 1993 Hislop *et al.* (51) achieved satisfactory results with annorganic xenografts in several maxillofacial applications, as interpositional graft, on-lay application, and post traumatic defects reconstruction. In two opportunities Froum *et al.* (52,53) demonstrated the success of maxillary sinus floor augmentation with xenografts, and further this type of graft procedure was demonstrated to have a long-term stability.

After the consolidation of the pioneer commercial brands in the xenografts market, new brands have entered in the industry, developing new purification techniques, innovations in micro and nano structural arrangements and different presentation forms of xenografts.

Physicochemical properties and purification

In the industry of biomaterials, we can find different approaches to handle with the cleaning phase of xeno or xeno-synthethic biomaterials, as stated in the historical line the main concerning is relative to the presence of other species proteins which has the potential to induce immunologic response in humans or disease development.
The first methods of purification consisted in simple mechanical fragmenting and physical exposition to low temperatures, as boiling or dry oven. This process removes the macro and visible organic constituents of animal bone, but not the collagen proteins which compound the bone matrix, or the cellular constituent (48,54,55).

Therefore, other purification methods were developed, as the ultra-high temperature treatment, in which the animal derived hydroxyapatite is treated with ultra-high temperatures, up to 1,000 ℃ (54,56). This method has been stated to eliminate even organic remnants in the microstructure of xenogenous bone, however the recent literature raises concerning about the loss of micro architecture. When the hydroxyapatite crystals, regardless the origin, are exposed to high temperatures it is supposed to occur a melting of the crystalline structure which lead to loss of mechanical resistance, surface hydrophily and porosity (56-58).

Otherwise, chemical treatment options also have been introduced as an alternative to ultra-high temperatures processing. Chemical processing originally consists of

submitting the animal derived bone, in particulate or block form, to several baths of alkaline solution, with increasing pH, most performed with NaOH baths (54). Those baths would be able not only to remove organic remnants as well as expose the intrinsic fibers and entrapped proteins in bone matrix, providing a bone without traces of donor genetic components. However, the complete removal of organic phase is hard to achieve, and recent studies demonstrated that most commercial brands present some level of organic remnants in their microstructure, as collagen fibers, cellular remnants or even animal RNA (49,59). One advantage of chemical processing is the preservation of the mechanical resistance of the xenografts and a well-defined porosity at micro and nano scale, as well as better moistening which could improve cellular and growth factors adhesion in material surface.

The combining of chemical and heat treatments lead to a complete organic phase removal, and also eliminate any trace of animal RNA, and the use of alkaline baths allows to use lower temperatures in the material purification process, thus the current most effective method combines alkaline solution baths with heating to up 300 ℃, and has been proved to be effective in removing even nucleic remnants, preserving in some amount the micro arrangement of hydroxyapatite crystals, which is as much important to keep the osteoconductive properties (54-57).

Laboratorial assays, biocompatibility and immunologic reactions

The clinical applications of xenografts were conducted empirically for at least two decades, and the good clinical results stimulated the development of basic and translational research on xenografts. Taylor *et al.* (60) demonstrated the cell adhesion and differentiation in the surface of allografts, xenografts and synthetic biomaterials *in vitro*, and found a higher presence of tartrate-resistant acid phosphatase (TRAP) + cells in the surface of DBBM, also the expression of type I collagen and noncollagenous proteins as osteopontin and bone sialoprotein were associated to a marked osteoclast activity.

Biocompatibility of xenografts was demonstrated by Matsumoto *et al.* (58), by seeding different mineral substrates with osteoblast like cells and evaluating cell proliferation on its surface. According to Matsumoto, the cell cultures exposed to bovine derived hydroxyapatite presents upregulated expression of collagen type I and osteocalcin in relation to plain cell culture used as control. Also, Mayr-Wohlfart *et al.* (61) demonstrated the potential of bioceramic and bioactive glasses onto inducing osteoblastic differentiation in a human osteoblastic-like cell line (SaOs2), which was also proved by Turhani *et al.* (62) in xenogenous hydroxyapatite derived from red algae.

The success of cellular assays using bone substitutes to evaluate cellular response also lead to a new field of application of xenografts as a scaffold for *in vitro* cellular growth and proliferation assays, and to the development of techniques for bone regeneration using stromal cells growth, as demonstrated by Krishnamurithy *et al.* (63).

Recently, the increase in the number of commercial brands of xeno and xeno-synthethic bone grafts, some *in vitro* studies were designed to compare the effectiveness of different commercial brands or bone graft presentations. Gehrke *et al.* (57) compared the bioactivity and physicochemical properties of sintered and non-sintered bovine bone blocks, and concluded both material present cellular adhesion and osteoconductivity, also concluded that the bioactivity is related to physicochemical properties of material. These findings corroborate previous studies which stated that porous size, density and wettability were direct related to *de novo* bone formation in bone substitutes.

With the broad comprehension of bone substitutes biocompatibility other studies aimed on a different focus, the inflammation process induced by xenografts implantation (64,65). Bovine bone substitutes were supposed to upregulate the expression of TNF-alfa and Interleukin-6, both pro inflammatory cytokines (66). However, the cellular reaction to bone grafts implantation could be a multinuclear giant cell mediated reaction rather than an osteoclastic one (67,68). A recent concept in literature states the macrophage modulated reaction could induce two different types of response at the implantation bed: a foreign body inflammatory reaction, or the biomaterial degradation followed by *de novo* bone formation (27,69,70). Therefore, the type of reaction could be determined by the physicochemical characteristics of the used biomaterial (65,70,71).

Animal studies

There are several *in vivo* studies using xenografts in the current literature, besides different species used the focus of animal studies can be classified in: *de novo* bone formation, biocompatibility and inflammatory response. The presentation forms and techniques used also vary, some studies aimed to compare commercial brands, while others evaluate the ideal proportion of mixed autogenous and

heterogenous bone.

Regarding bone *de novo* formation Yaedú *et al.* (72) compared histological and histometric property of osteoconductive bovine bone compound with phosphate beta tricalcium implanted in critical size defects in rat calvaria and they noticed that both materials experimented feature osteoconductive properties and can be used clinically for filling cavities. Ge *et al.* (73) tested the differences between grafts in surgically created defects in the dogs mandible to evaluate bone formation, their results for blood clot (no graft), autogenous bone graft, demineralized bovine bone with porcine collagen membrane and autogenous bone plus demineralized bovine bone in proportion 1:1 were similar. Abou Fadel *et al.* (26) realized a study to evaluate bone regeneration and residual grafts in critical size calvaria defects in rats, grafted with either a deproteinized bovine bone mineral alone or in combination with a single or double layer of native bilayer collagen membrane, and found better results in a single layer collagen membrane group to both analyzed aspects.

At the aspect of immunologic response to bone substitutes the most recent investigation field is the macrophage polarization, a concept introduced by Barbeck *et al.* after assessing *in vivo* response to high temperature sintered xenografts (74). The subcutaneous model to study inflammatory reaction to biomaterials in animals was recently spread in scientific literature and allows to determine the relevance of multinuclear giant cell into graft incorporation. Briefly, according to Rolvien *et al.* (27) the macrophages cans react by two pathways, the M1-like phenotype, which leads to encapsulation and graft failure, or the M2-like phenotype, which evolves material degradation and tissue regeneration.

In attempt to compare the effectiveness of equine and bovine derived bone substitutes to autologous bone Moest *et al.* (75) performed an experimental "critical size defect" model at the frontal skull area of pigs. Within the xenogeneic groups, significantly higher *de novo* bone formation could be observed for the equine bone group after 30 days. By 60 days after defect preparation, no statistically significant difference was observed between the bovine and equine group, whereas *de novo* bone formation was higher in the bovine bone group. After 60 days, no significant difference concerning connective tissue proportions or statistically significant differences concerning the remaining residual bone substitute material between xenogeneic groups was observed (75).

Animal studies were also used to evaluate xenografts in different alveolar ridge preservation techniques, for example Munhoz *et al.* (76) realized an experimental study in rabbits to evaluate the influence of an annorganic xenograft fill on the maintenance of alveolar bone height, bone density and the osseointegration course following the insertion of titanium implants. They created mandibular bone defects like alveolar sockets and grafted with ABB, maintaining a control group with the natural healing process of the defect. Later, titanium implants were installed. The results shown that the use of a xenograft prior to a titanium implant insertion did not interfere with the course of its osseointegration in rabbit's mandible (76).

Clinical applications

Most of clinical studies evaluating bone substitutes and grafting techniques are retrospective studies or case series. In general, biomaterials clinical research aimed to point which are the best material, evaluating the graft stability, implant success, bone new formation and bone density. The methods used to evaluate the success of bone grafting procedures using xenografts vary, but histological analysis and CT scan are the most common methods. We can divide clinical studies according to the augmentation technique proposed, as socket preservation, guided bone regeneration (GBR), sinus lift, vertical/horizontal ridge augmentation or osteotomy gap filling.

In a study to evaluate the long-term influence of xenogeneic grafts on bone crestal height and radiographic density following extraction of teeth, Andrade Munhoz *et al.* (77) created a control group with only natural healing process after tooth extraction and a test group where the extractions sockets were filled with inorganic bovine bone. The authors conclude that, in this case, the bone graft did not change bone crestal height and bone radiographic density in the long term.

As in the laboratory studies, to clinical analyses some authors hypothesize there are some differences in the xenografts' behaviors according to processing and fabrication methods. Block (78) showed some hypotheses, not confirmed, for a randomized study about differences between xenografts processing. According to the author, the method for processing xenograft bone can affect its resorption characteristics and its osteoconduction. Xenografts heated at a lower temperature have a faster resorption rate *in situ* compared with xenografts heated at higher temperatures. This might be important to consider when choosing a xenograft for a specific clinical indication.

In disagreement with most studies available in the literature, Serrano Méndez *et al.* (25) proceeded a comparative study with demineralized freeze-dried cortical bone allografts and deproteinized bovine bone mineral xenografts embedded in a 10% collagen matrix, with 20 randomized patients and concluded that there are no statistically significant different outcomes of the grafting material used for the bone augmentation. Hence, it is postulated that both grafting materials are suitable for the preservation of the alveolar ridge (25).

Whilst the use of autogenous bone provides the unique benefits of osteoinductivity and osteogenic potential. Extensive remodelling may occur when it is used as the sole material for sinus augmentation. This has been demonstrated to lead to undesirable volumetric changes after grafting in the first year.

Cassetta *et al.* (8) accomplished a histological, histomorphometrical and clinical human study to compare the outcomes, after a 2-month healing period of autologous bone, porcine bone, and a 50:50 mixture of the two in maxillary sinus augmentation procedures. The histomorphometry analysis revealed comparable percentages of newly formed bone, marrow spaces, and residual grafted material in the three groups. The clinical and histological results indicated that porcine bone alone or in combination with autologous bone are biocompatible and osteoconductive materials and can be successfully used in sinus augmentation procedures (8).

A similar comparative study in human maxillary sinus augmentation was performed by Schmitt *et al.* (79), but the grafts utilized were ABB (Bio-Oss®, Geistlich Pharma North America, Inc., Princeton, NJ, USA) and ABB plus autologous bone with a ratio of 1/1. The results founded were similar too. The effect of adding autologous bone with its osteoinductive properties has no beneficial effect on newly formed bone. Due to its limited biodegradation, it is hypothesized that addition of Bio-Oss to autogenous bone graft or the sole use of Bio-Oss is an advantage for bone preservation (79,80).

To identify the most effective biomaterials producing higher new bone formation and lower residual graft and connective tissue following maxillary sinus augmentation, Al-Moraissi *et al.* (81) searched to identify randomized controlled trials published until March 2018, evaluating histomorphometry outcomes after maxillary sinus augmentation. Predictor variables were autogenous bone, allografts, xenografts, alloplastic bone and grafts combined with autologous platelet concentrates/recombinant growth factors, mesenchymal stem cells, or recombinant bone morphogenetic proteins. They concluded that, in fact, autogenous bone showed the best performance only when the healing time was shorter than 6 months, while for a longer healing time most materials achieved similar histomorphometry results. The clinical implication of this finding is that grafting with autogenous bone is recommended when implant rehabilitation is planned within 6 months of the grafting procedure. Also, the addition of autogenous bone to growth factors, or mesenchymal cells to any biomaterial may increase the healing rate.

Park *et al.* (82) in a retrospective study with patients who received vertical ridge augmentation, evaluated radiographic outcomes in terms of the augmented height and its maintenance following vertical ridge augmentation procedures using collagen membrane and different types of materials including DBBM and synthetic biphasic calcium phosphate. The clinical findings of this study suggest that the alveolar ridge can be vertically augmented using either allogenous bone block or particulate bone substitute. However, they required a longer healing period to ensure dimensional stability compared to using autogenous bone block (82).

Lai *et al.* (83) published a study with the goal to compare the histologic and clinical outcomes of ridge preservation using bovine and porcine xenografts in 44 patients. The current findings indicate that there are no significant differences in vital bone formation, residual graft particles, and connective tissue between the groups after 18 to 20 weeks of wound healing. There were no significant differences in the clinical dimensional changes of the alveolar ridge between the groups.

Tetè *et al.* (84) proceeded with an increase of the posterior region of maxilla in 20 patients needing pre-prosthetic rehabilitation and two groups were created to compare the molecular events switched on by autologous or heterologous bone graft insertion. Ten patients underwent maxillary sinus augmentation procedure with a bone substitute of equine origin (BioBone®, Osteoconductor Mix, BioSAF IN S.r.l., Ancona, Italy) and ten patients received an onlay bone graft and a maxillary sinus augmentation procedure with bone obtained from the parietal region of the calvaria. Such results host bone tissue, undergoing regenerative phenomena, positively reacted to the placement of both biomaterials. In particular, the equine-derived biomaterial shows good characteristics, in terms of both clinical and microscopic integration. However, at the same experimental time, sites treated with autologous

bone clearly show a better organization, which could ensure a better primary stability to the implant and a higher predictability of the implant-prosthetic rehabilitation (84).

By other side Pistilli *et al.* (19) found high failure rate (up to 50%) and graft loss using the equine bone in a clinical trial comparing the equine derived xenogeneic bone block with autogenous bone of mandibular ramus or iliac crest, of failure. In autogenous group all procedures performed in maxilla were successful. Furthermore, previous clinical studies also have not found promising results of equine bone for ridge augmentation procedures (21).

On this behalf, the clinical use of DBBM grafts is not free of complications, suture dehiscence, graft exposure, infection, graft resorption, granular reaction and partial or total graft loss are some of the most often reported complications in DBBM graft procedures (7,85). The graft exposure to the oral cavity leads to other events which may impair the incorporation. Complications can occur in early or late stages of healing, but the early exposure is related with soft tissue injuries, inadequate soft tissue management or suture dehiscence (86,87). In cases using particulate bone grafts the exposure leads to infection and graft loss (86,88,89). When using bone blocks, the exposures can be managed with disinfection, graft remodeling, soft tissue graft and/or new sutures (24,90,91).

Another interesting aspect to analyze is the size of the graft, de Molon *et al.* (92) investigated sinus floor augmentation with two different particle sizes of demineralized bovine bone mineral by means of histological and immunohistochemical analysis, histomorphometry analysis revealed no statistically significant difference in the percentage of biomaterial, newly formed bone or connective tissue between the small and large-sized particle groups. Immunohistochemical analysis did not reveal differences in the expression of protein osteocalcin, vascular endothelial growth factor or tartrate resistant acid phosphatase. No complications were observed during the entire healing period and the survival rate of the implants was 100%.

Innovations and new concepts have also been incorporated to the use of DBBM grafts in the recent 10 years, the use of blood concentrates is one the most promising improvements to the technique of DBBM grafts. Blood concentrates such as platelet-rich fibrin (PRF), have been used in many fields of regenerative medicine, often in combination with particulate grafts as an agglutinant or as an adjuvant membrane in primary covering and stability promoting (93-95). Most of research about platelet-rich plasma (PRP) and PRF use these biomaterials incorporated in bone grafts, to enhance new bone formation.

The technique for DBBM usage in grafting procedures has also been a matter of investigation, rather its use alone can be vantageous there is also benefits in mixing DBBM with autologous bone. Mordenfeld *et al.* (96) performed a split-mouth study to evaluate horizontal ridge augmentation with DBBM, but using a combination of particulate DBBM with particulate autogenous bone in different proportions, 60:40 and 90:10, achieving a horizontal gain of 82% of initial volume and resorption rate between 27% and 49%. The combination of both grafts in the same site seems to enhance properties of xenogenous graft, and minimize the limitations of autogenous bone alone, confirming in clinical study some of the concepts developed in animal assay.

Conclusions

The use of xenogeneic bones substitutes is not new, however, most of its applications were based on clinical experience and success. The current research about xenografts reverted the mindset to bring light for the biological features and mechanisms which lead to biomaterial clinical success. The laboratory research now supports the clinical use, and the knowledge of basic cellular processes allow the development of new biomaterials with physicochemical properties closer to the ideal bone substitute.

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References

1. Pelegrine AA, Teixeira ML, Sperandio M, et al. Can bone marrow aspirate concentrate change the mineralization pattern of the anterior maxilla treated with xenografts? A preliminary study. Contemp Clin Dent 2016;7:21-6.
2. Barone A, Aldini NN, Fini M, et al. Xenograft versus extraction alone for ridge preservation after tooth removal: a clinical and histomorphometric study. J Periodontol 2008;79:1370-7.
3. Brugnami F, Then PR, Moroi H, et al. GBR in human extraction sockets and ridge defects prior to implant placement: clinical results and histologic evidence of osteoblastic and osteoclastic activities in DFDBA. Int J Periodontics Restorative Dent 1999;19:259-67.
4. Esposito M, Grusovin MG, Felice P, et al. The efficacy of horizontal and vertical bone augmentation procedures for dental implants - a Cochrane systematic review. Eur J Oral Implantol 2009;2:167-84.
5. Stopa Z, Siewert-Gutowska M, Abed K, et al. Evaluation of the Safety and Clinical Efficacy of Allogeneic Bone Grafts in the Reconstruction of the Maxilla and Mandible. Transplant Proc 2018;50:2199-201.
6. Cardoso CL, Curra C, Santos PL, et al. Current considerations on bone substitutes in maxillary sinus lifting. Revista Clínica de Periodoncia, Implantología y Rehabilitación Oral 2016;9:102-7.
7. Chiapasco M, Casentini P, Zaniboni M. Bone augmentation procedures in implant dentistry. Int J Oral Maxillofac Implants 2009;24 Suppl:237-59.
8. Cassetta M, Perrotti V, Calasso S, et al. Bone formation in sinus augmentation procedures using autologous bone, porcine bone, and a 50 : 50 mixture: a human clinical and histological evaluation at 2 months. Clin Oral Implants Res 2015;26:1180-4.
9. Clementini M, Morlupi A, Agrestini C, et al. Success rate of dental implants inserted in autologous bone graft regenerated areas: a systematic review. Oral Implantol (Rome) 2011;4:3-10.
10. Sbordone L, Toti P, Menchini-Fabris GB, et al. Volume changes of autogenous bone grafts after alveolar ridge augmentation of atrophic maxillae and mandibles. Int J Oral Maxillofac Surg 2009;38:1059-65.
11. Spin-Neto R, Stavropoulos A, Coletti FL, et al. Graft incorporation and implant osseointegration following the use of autologous and fresh-frozen allogeneic block bone grafts for lateral ridge augmentation. Clin Oral Implants Res 2014;25:226-33.
12. Sbordone C, Toti P, Guidetti F, et al. Volume changes of iliac crest autogenous bone grafts after vertical and horizontal alveolar ridge augmentation of atrophic maxillas and mandibles: a 6-year computerized tomographic follow-up. J Oral Maxillofac Surg 2012;70:2559-65.
13. Pereira RS, Pavelski MD, Griza GL, et al. Prospective evaluation of morbidity in patients who underwent autogenous bone-graft harvesting from the mandibular symphysis and retromolar regions. Clin Implant Dent Relat Res 2019;21:753-7.
14. Nkenke E, Neukam FW. Autogenous bone harvesting and grafting in advanced jaw resorption: morbidity, resorption and implant survival. Eur J Oral Implantol 2014;7 Suppl 2:S203-17.
15. Barone A, Toti P, Menchini-Fabris GB, et al. Early volumetric changes after vertical augmentation of the atrophic posterior mandible with interpositional block graft versus onlay bone graft: A retrospective radiological study. J Craniomaxillofac Surg 2017;45:1438-47.
16. Carlsen A, Gorst-Rasmussen A, Jensen T. Donor site morbidity associated with autogenous bone harvesting from the ascending mandibular ramus. Implant Dent 2013;22:503-6.
17. Papageorgiou SN, Papageorgiou PN, Deschner J, et al. Comparative effectiveness of natural and synthetic bone grafts in oral and maxillofacial surgery prior to insertion

of dental implants: Systematic review and network meta-analysis of parallel and cluster randomized controlled trials. J Dent 2016;48:1-8.
18. Jensen SS, Broggini N, Hjørting-Hansen E, et al. Bone healing and graft resorption of autograft, anorganic bovine bone and beta-tricalcium phosphate. A histologic and histomorphometric study in the mandibles of minipigs. Clin Oral Implants Res 2006;17:237-43.
19. Pistilli R, Felice P, Piatelli M, et al. Blocks of autogenous bone versus xenografts for the rehabilitation of atrophic jaws with dental implants: preliminary data from a pilot randomised controlled trial. Eur J Oral Implantol 2014;7:153-71.
20. Motamedian SR, Khojaste M, Khojasteh A. Success rate of implants placed in autogenous bone blocks versus allogenic bone blocks: A systematic literature review. Ann Maxillofac Surg 2016;6:78-90.
21. Felice P, Marchetti C, Iezzi G, et al. Vertical ridge augmentation of the atrophic posterior mandible with interpositional bloc grafts: bone from the iliac crest vs. bovine anorganic bone. Clinical and histological results up to one year after loading from a randomized-controlled clinical trial. Clin Oral Implants Res 2009;20:1386-93.
22. Veis A, Dabarakis N, Koutrogiannis C, et al. Evaluation of Vertical Bone Regeneration Using Block and Particulate Forms of Bio-Oss Bone Graft: A Histologic Study in the Rabbit Mandible. J Oral Implantol 2015;41:e66-72.
23. Block MS, Ducote CW, Mercante DE. Horizontal augmentation of thin maxillary ridge with bovine particulate xenograft is stable during 500 days of follow-up: preliminary results of 12 consecutive patients. J Oral Maxillofac Surg 2012;70:1321-30.
24. Schwarz F, Mihatovic I, Ghanaati S, et al. Performance and safety of collagenated xenogeneic bone block for lateral alveolar ridge augmentation and staged implant placement. A monocenter, prospective single-arm clinical study. Clin Oral Implants Res 2017;28:954-60.
25. Serrano Méndez CA, Lang NP, Caneva M, et al. Comparison of allografts and xenografts used for alveolar ridge preservation. A clinical and histomorphometric RCT in humans. Clin Implant Dent Relat Res 2017;19:608-15.
26. Abou Fadel R, Samarani R, Chakar C. Guided bone regeneration in calvarial critical size bony defect using a double-layer resorbable collagen membrane covering a xenograft: a histological and histomorphometric study in rats. Oral Maxillofac Surg 2018;22:203-13.
27. Rolvien T, Barbeck M, Wenisch S, et al. Cellular Mechanisms Responsible for Success and Failure of Bone Substitute Materials. Int J Mol Sci 2018;19:2893.
28. Nasr HF, Aichelmann-Reidy ME, Yukna RA. Bone and bone substitutes. Periodontol 2000 1999;19:74-86.
29. Gruskin E, Doll BA, Futrell FW, et al. Demineralized bone matrix in bone repair: history and use. Adv Drug Deliv Rev 2012;64:1063-77.
30. Macewen W. Observations concerning transplantation of bone. Illustrated by a case of inter-human osseous transplantation, whereby over two-thirds of the shaft of a humerus was restored. Proc R Soc London 1881;32:232-47.
31. Senn N. On the healing of aseptic bone cavities by implantation of antiseptic decalcified bone. Ann Surg 1889;10:352-68.
32. Porritt N. A Case of Successful Sponge-Grafting. Edinb Med J 1882;27:975-6.
33. Pryor LS, Gage E, Langevin CJ, et al. Review of bone substitutes. Craniomaxillofac Trauma Reconstr 2009;2:151-60.
34. Kolk A, Handschel J, Drescher W, et al. Current trends and future perspectives of bone substitute materials - from space holders to innovative biomaterials. J Craniomaxillofac Surg 2012;40:706-18.
35. Orell S. Surgical bone grafting with "os purum"," os novum", and "boiled bone". JBJS 1937;19:873-85.
36. Perry CR. Bone repair techniques, bone graft, and bone graft substitutes. Clin Orthop Relat Res 1999;(360):71-86.
37. Hurley LA, Zeier FG, Stinchfield FE. Anorganic bone grafting. Clinical experiences with heterografts processed by ethylenediamine extraction. Am J Surg 1960;100:12-21.
38. Hurley LA, Losee FL. Anorganic bone; chemistry, anatomy, and biological reactions. Mil Med 1957;121:101-4.
39. Anderson KJ, Lecocq JF, Dingwall JA, et al. Processed heterogenous bone; a basic scientific study with preliminary clinical trails in humans. JAMA 1965;193:377-80.
40. Hürzeler MB, Kirsch A, Ackermann KL, et al. Reconstruction of the severely resorbed maxilla with dental implants in the augmented maxillary sinus: a 5-year clinical investigation. Int J Oral Maxillofac Implants 1996;11:466-75.
41. Browaeys H, Bouvry P, De Bruyn H. A literature review on biomaterials in sinus augmentation procedures. Clin Implant Dent Relat Res 2007;9:166-77.
42. Nery EB, LeGeros RZ, Lynch KL, et al. Tissue response to biphasic calcium phosphate ceramic with different ratios of HA/beta TCP in periodontal osseous defects. J Periodontol 1992;63:729-35.
43. Fukuta K, Har-Shai Y, Collares MV, et al. Comparison of inorganic bovine bone mineral particles with porous

hydroxyapatite granules and cranial bone dust in the reconstruction of full-thickness skull defect. J Craniofac Surg 1992;3:25-9.
44. Wenz B, Oesch B, Horst M. Analysis of the risk of transmitting bovine spongiform encephalopathy through bone grafts derived from bovine bone. Biomaterials 2001;22:1599-606.
45. Sogal A, Tofe AJ. Risk assessment of bovine spongiform encephalopathy transmission through bone graft material derived from bovine bone used for dental applications. J Periodontol 1999;70:1053-63.
46. Kim Y, Rodriguez AE, Nowzari H. The Risk of Prion Infection through Bovine Grafting Materials. Clin Implant Dent Relat Res 2016;18:1095-102.
47. Di Stefano DA, Artese L, Iezzi G, et al. Alveolar ridge regeneration with equine spongy bone: a clinical, histological, and immunohistochemical case series. Clin Implant Dent Relat Res 2009;11:90-100.
48. Bow A, Anderson DE, Dhar M. Commercially available bone graft substitutes: the impact of origin and processing on graft functionality. Drug Metab Rev 2019;51:533-44.
49. Ghanaati S, Barbeck M, Booms P, et al. Potential lack of "standardized" processing techniques for production of allogeneic and xenogeneic bone blocks for application in humans. Acta Biomater 2014;10:3557-62.
50. Scheer P, Boyne PJ. Maintenance of alveolar bone through implantation of bone graft substitutes in tooth extraction sockets. J Am Dent Assoc 1987;114:594-7.
51. Hislop WS, Finlay PM, Moos KF. A preliminary study into the uses of anorganic bone in oral and maxillofacial surgery. Br J Oral Maxillofac Surg 1993;31:149-53.
52. Froum SJ, Wallace S, Cho SC, et al. Histomorphometric comparison of different concentrations of recombinant human bone morphogenetic protein with allogeneic bone compared to the use of 100% mineralized cancellous bone allograft in maxillary sinus grafting. Int J Periodontics Restorative Dent 2013;33:721-30.
53. Froum SJ, Tarnow DP, Wallace SS, et al. Sinus floor elevation using anorganic bovine bone matrix (OsteoGraf/N) with and without autogenous bone: a clinical, histologic, radiographic, and histomorphometric analysis--Part 2 of an ongoing prospective study. Int J Periodontics Restorative Dent 1998;18:528-43.
54. Perić Kačarević Z, Kavehei F, Houshmand A, et al. Purification processes of xenogeneic bone substitutes and their impact on tissue reactions and regeneration. Int J Artif Organs 2018;41:789-800.
55. Barbeck M, Jung O, Xiong X, et al. Balancing Purification and Ultrastructure of Naturally Derived Bone Blocks for Bone Regeneration: Report of the Purification Effort of Two Bone Blocks. Materials (Basel) 2019;12:3234.
56. Goller G, Oktar FN, Agathopoulos S, et al. ''Effect of sintering temperature on mechanical and microstructural properties of bovine hydroxyapatite (BHA)''. Journal of Sol-Gel Science and Technology 2006;37:111-5.
57. Gehrke SA, Mazón P, Pérez-Díaz L, et al. Study of Two Bovine Bone Blocks (Sintered and Non-Sintered) Used for Bone Grafts: Physico-Chemical Characterization and In Vitro Bioactivity and Cellular Analysis. Materials (Basel) 2019;12:452.
58. Matsumoto T, Kawakami M, Kuribayashi K, et al. Effects of sintered bovine bone on cell proliferation, collagen synthesis, and osteoblastic expression in MC3T3-E1 osteoblast-like cells. J Orthop Res 1999;17:586-92.
59. Orlowska A, Al-Maawi S, Brozovic J, et al. Xeno-synthetic bone block includes cellular remnants: Acceptable components or lack of purification? Int J Growth Factors Stem Cells Dent 2018;1:70-3.
60. Taylor JC, Cuff SE, Leger JP, et al. In vitro osteoclast resorption of bone substitute biomaterials used for implant site augmentation: a pilot study. Int J Oral Maxillofac Implants 2002;17:321-30.
61. Mayr-Wohlfart U, Fiedler J, Günther KP, et al. Proliferation and differentiation rates of a human osteoblast-like cell line (SaOS-2) in contact with different bone substitute materials. J Biomed Mater Res 2001;57:132-9.
62. Turhani D, Weissenböck M, Watzinger E, et al. Invitro study of adherent mandibular osteoblast-like cells on carrier materials. Int J Oral Maxillofac Surg 2005;34:543-50.
63. Krishnamurithy G, Murali MR, Hamdi M, et al. Characterization of bovine-derived porous hydroxyapatite scaffold and its potential to support osteogenic differentiation of human bone marrow derived mesenchymal stem cells. Ceram Int 2014;40:771-7.
64. Anderson JM, Rodriguez A, Chang DT. Foreign body reaction to biomaterials. Semin Immunol 2008;20:86-100.
65. Barbeck M, Motta A, Migliaresi C, et al. Heterogeneity of biomaterial-induced multinucleated giant cells: Possible importance for the regeneration process? J Biomed Mater Res A 2016;104:413-8.
66. Amerio P, Vianale G, Reale M, et al. The effect of deproteinized bovine bone on osteoblast growth factors and proinflammatory cytokine production. Clin Oral Implants Res 2010;21:650-5.
67. Barbeck M, Udeabor SE, Lorenz J, et al. Induction of

multinucleated giant cells in response to small sized bovine bone substitute (Bio-Oss™) results in an enhanced early implantation bed vascularization. Ann Maxillofac Surg 2014;4:150-7.
68. Barbeck M, Booms P, Unger R, et al. Multinucleated giant cells in the implant bed of bone substitutes are foreign body giant cells-New insights into the material-mediated healing process. J Biomed Mater Res A 2017;105:1105-11.
69. Fujioka-Kobayashi M, Marjanowski SD, Kono M, et al. In Vitro Comparison of Macrophage Polarization and Osteoblast Differentiation Potentials between Granules and Block Forms of Deproteinized Bovine Bone Mineral. Materials (Basel) 2020;13:2682.
70. Fujioka-Kobayashi M, Katagiri H, Kono M, et al. The impact of the size of bone substitute granules on macrophage and osteoblast behaviors in vitro. Clin Oral Investig 2021;25:4949-58.
71. Shi M, Wang C, Wang Y, et al. Deproteinized bovine bone matrix induces osteoblast differentiation via macrophage polarization. J Biomed Mater Res A 2018;106:1236-46.
72. Yaedú RYF, de Carvalho PSP, Ferreira Júnior O, et al. Morfologic osteoconduction evaluation of Gen-Phos and Gen-Mix in rat calvaria. Dent Press Implantol 2012;6:97-104.
73. Ge J, Yang C, Wang Y, et al. Comparison of different grafting materials for treatment of bone defect distal to the molar in canine. Clin Implant Dent Relat Res 2018;20:444-54.
74. Barbeck M, Udeabor S, Lorenz J, et al. High-Temperature Sintering of Xenogeneic Bone Substitutes Leads to Increased Multinucleated Giant Cell Formation: In Vivo and Preliminary Clinical Results. J Oral Implantol 2015;41:e212-22.
75. Moest T, Wehrhan F, Lutz R, et al. Extra-oral defect augmentation using autologous, bovine and equine bone blocks: A preclinical histomorphometrical comparative study. J Craniomaxillofac Surg 2015;43:559-66.
76. Munhoz EA, Bodanezi A, Cestari TM, et al. Long-term rabbits bone response to titanium implants in the presence of inorganic bovine-derived graft. J Biomater Appl 2012;27:91-8.
77. Andrade Munhoz E, Bodanezi A, Ferreira Junior O, et al. Bone crestal height and bone density after third-molar extraction and grafting: a long-term follow-up study. Clin Oral Investig 2011;15:123-6.
78. Block MS. The Processing of Xenografts Will Result in Different Clinical Responses. J Oral Maxillofac Surg 2019;77:690-7.
79. Schmitt CM, Moest T, Lutz R, et al. Anorganic bovine bone (ABB) vs. autologous bone (AB) plus ABB in maxillary sinus grafting. A prospective non-randomized clinical and histomorphometrical trial. Clin Oral Implants Res 2015;26:1043-50.
80. Mordenfeld A, Hallman M, Johansson CB, et al. Histological and histomorphometrical analyses of biopsies harvested 11 years after maxillary sinus floor augmentation with deproteinized bovine and autogenous bone. Clin Oral Implants Res 2010;21:961-70.
81. Al-Moraissi EA, Alkhutari AS, Abotaleb B, et al. Do osteoconductive bone substitutes result in similar bone regeneration for maxillary sinus augmentation when compared to osteogenic and osteoinductive bone grafts? A systematic review and frequentist network meta-analysis. Int J Oral Maxillofac Surg 2020;49:107-20.
82. Park YH, Choi SH, Cho KS, et al. Dimensional alterations following vertical ridge augmentation using collagen membrane and three types of bone grafting materials: A retrospective observational study. Clin Implant Dent Relat Res 2017;19:742-9.
83. Lai VJ, Michalek JE, Liu Q, et al. Ridge preservation following tooth extraction using bovine xenograft compared with porcine xenograft: A randomized controlled clinical trial. J Periodontol 2020;91:361-8.
84. Tetè S, Vinci R, Zizzari VL, et al. Maxillary sinus augmentation procedures through equine-derived biomaterial or calvaria autologous bone: immunohistochemical evaluation of OPG/RANKL in humans. Eur J Histochem 2013;57:e10.
85. Checchi V, Gasparro R, Pistilli R, et al. Clinical Classification of Bone Augmentation Procedure Failures in the Atrophic Anterior Maxillae: Esthetic Consequences and Treatment Options. Biomed Res Int 2019;2019:4386709.
86. Lim G, Lin GH, Monje A, et al. Wound Healing Complications Following Guided Bone Regeneration for Ridge Augmentation: A Systematic Review and Meta-Analysis. Int J Oral Maxillofac Implants 2018;33:41–50.
87. Jensen AT, Jensen SS, Worsaae N. Complications related to bone augmentation procedures of localized defects in the alveolar ridge. A retrospective clinical study. Oral Maxillofac Surg 2016;20:115-22.
88. Li J, Wang HL. Common implant-related advanced bone grafting complications: classification, etiology, and management. Implant Dent 2008;17:389-401.
89. de Azambuja Carvalho PH, Dos Santos Trento G, Moura LB, et al. Horizontal ridge augmentation using

xenogenous bone graft-systematic review. Oral Maxillofac Surg 2019;23:271-9.
90. Aloy-Prósper A, Peñarrocha-Oltra D, Peñarrocha-Diago M, et al. The outcome of intraoral onlay block bone grafts on alveolar ridge augmentations: a systematic review. Med Oral Patol Oral Cir Bucal 2015;20:e251-8.
91. Schwartz-Arad D, Levin L. Intraoral autogenous block onlay bone grafting for extensive reconstruction of atrophic maxillary alveolar ridges. J Periodontol 2005;76:636-41.
92. de Molon RS, Magalhaes-Tunes FS, Semedo CV, et al. A randomized clinical trial evaluating maxillary sinus augmentation with different particle sizes of demineralized bovine bone mineral: histological and immunohistochemical analysis. Int J Oral Maxillofac Surg 2019;48:810-23.
93. Simonpieri A, Del Corso M, Sammartino G, et al. The relevance of Choukroun's platelet-rich fibrin and metronidazole during complex maxillary rehabilitations using bone allograft. Part II: implant surgery, prosthodontics, and survival. Implant Dent 2009;18:220-9.
94. Ghanaati S, Al-Maawi S, Conrad T, et al. Biomaterial-based bone regeneration and soft tissue management of the individualized 3D-titanium mesh: An alternative concept to autologous transplantation and flap mobilization. J Craniomaxillofac Surg 2019;47:1633-44.
95. Ghanaati S, Herrera-Vizcaino C, Al-Maawi S, et al. Fifteen Years of Platelet Rich Fibrin in Dentistry and Oromaxillofacial Surgery: How High is the Level of Scientific Evidence? J Oral Implantol 2018;44:471-92.
96. Mordenfeld A, Johansson CB, Albrektsson T, et al. A randomized and controlled clinical trial of two different compositions of deproteinized bovine bone and autogenous bone used for lateral ridge augmentation. Clin Oral Implants Res 2014;25:310-20.

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